Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.033

RESEARCH ARTICLE

Characterization of *Salmonella* Enterica Isolated from Poultry Hatcheries and Commercial Broiler Chickens

Awad A Shehata[1], Shereen Basiouni[2], Alaa Abd Elrazek[1], Hesham Sultan[1], Mohamed Sabry Abd Elraheam Elsayed[3]*, Reda Tarabees[3], Shaimaa Talat[1], Ibrahim Moharam[1], Ahmed Said[1], Walaa Atia Mohsen[1], Tamer Roshdey[4] and Monika Krüger[5]

[1]Birds and Rabbit Diseases Department, Faculty of Veterinary Medicine, Sadat City University, Egypt; [2]Clinical Pathology Department, Faculty of Veterinary Medicine, Benha University, Benha, Egypt; [3]Department of Bacteriology and Mycology, Faculty of Veterinary Medicine, Sadat City University, Minoufiya, Egypt; [4]Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Sadat City, Menoufia, Egypt;[5]Albrecht Daniel Thaer-Institute of Agronomy, Faculty of Veterinary Medicine, Leipzig University, Germany
*Corresponding author: mohammedelsayed529@yahoo.com; mohamed.sabry@vet.usc.edu.eg

ARTICLE HISTORY (18-379)

Received: October 04, 2018
Revised: November 26, 2018
Accepted: December 20, 2018
Published online: March 07, 2019

Key words:
Antimicrobial resistance
Pathogenicity
Poultry hatcheries
Salmonella
Virulence genes

A B S T R A C T

Salmonella, is a serious pathogen causing disastrous losses in the poultry production and dangerous human infections. This study was aimed to identify *Salmonella* serotypes and to detect the prominent virulence genes and antimicrobial resistance of the obtained serotypes from samples collected from dead chicken embryos, dead duck embryos and commercial broilers. The pathogenicity of some serotypes as (*Salmonella* Pullorum, *Salmonella* Enteritidis, and *Salmonella* Typhimurium) was investigated in one-day-old commercial layer chicks. A total of 180 cases were collected, after isolation 18 *Salmonella* isolates were reported to be as follows 7/100(7%), 5/40(12.5%) and 6/40(15%) in broilers, dead chickens, and dead duck embryos, respectively. The most prevalent serotypes were *S.* Enteritidis, *S.* Typhimurium, *S.* Pullorum, *S.* Vejle, *S.* Amsterdam, *S.* Infantis, *S.* Petersburg, and *S.* Atakpame. The distribution patterns of virulence genes *iro*N, *cdt*B, *spa*N, *inv*A, and *org*A, expressed as follows; 17/18(94.4%), 15/18(83.3%), 14/18(77.7%), 13/18(72.2%), and 12/18(66.7%) of serotypes, respectively. Added to that, the *sip*V, *Ipf*C, *sop*B, *prg*H, and *sit*C virulence genes were amplified in 7/18(38.8%), 7/18(38.8%), 7/18(38.8%), 5/18(27.7%) and 3/18(16.6%) of isolates respectively. Most of isolates 16/18 (88.9%) expressed multiple antibiotic resistances (MAR) indices ranged from ≥0.3 ≤1. Based on the pathogenicity testing, *S.* Pullorum was the most pathogenic due to the clear signs and a mortality rate of 35%. Hence, the genotypic characterization and continuous monitoring of antimicrobial resistance of *Salmonella* from poultry are of public health concern to implement effective control strategies against this notorious pathogen.

To Cite This Article: Shehata AA, Basiouni S, Elrazek AA, Sultan H, Tarabees R, Elsayed MSAE, Talat S, Moharam I, Said A, Mohsen WA and Krüger M, 2019. Characterization of *Salmonella* Enterica isolated from poultry hatcheries and commercial broiler chickens. Pak Vet J, 39(4): 515-520. http://dx.doi.org/10.29261/pakvetj/2019.033

INTRODUCTION

Salmonella is a Gram-negative, rod-shaped, intracellular human and animal pathogen and a major public health concern (Chlebicz and Śliżewska, 2018). To date, the number of described *Salmonella* enterica serovars has exceeded 2500 (Dieckmann and Malorny, 2011). Certain serovars are host restricted, while others have a broad host range (Grassl and Finlay, 2008). There are two main pathogenic groups of the genus *Salmonella*. The first group is host adapted and produces a systemic, typhoid disease. This group includes the serovars S. Gallinarum and S. Pullorum, which lead to fowl typhoid and pullorum disease in chicken respectively (Chao *et al.*, 2007). Clinical signs of Pullorum disease are principally noticed in young aged chicks that show depression, lost appetite, distressed respiration, diarrhoea with caseous core, and high post hatching offspring deaths. Moreover, the symptoms in laying hens characterized by decreased egg productivity, lowered fertility, and reduced hatchability

rate (Hameed *et al.,* 2014). The second pathogenic group represents zoonotic *Salmonella* serovars that cause food poisoning in humans and can cause disease in immuno-compromised hosts or after viral infections (Barrow, 2007). This group includes serovars that are frequently identified from human cases like *Salmonella enterica* serotypes Agona, Enteritidis, Hadar, Infantis, Newport, Typhimurium, and Virchow (Hendriksen *et al.*, 2011).

Salmonella is introduced into eggs via both vertical and horizontal transmission routes. Consequently, *Salmonella* affects fertility, harms embryonic development, causes early embryonic mortality and infection to other chicks and humans (Pande *et al.,* 2016).

The pervasive use of antibiotics in domestic animals and the poultry industry has resulted in drug-resistant bacteria that are transferred to humans (Franco *et al.,* 2015). This study aimed to identify the most prevalent *Salmonella* serotypes from dead embryos, duck hatcheries, and commercial broilers and genotypic detection of the distribution pattern of the virulence genes harbored by these serotypes. Moreover, we performed antibiogram susceptibility testing to screen for the antimicrobial resistance patterns of the obtained isolates. Pathogenicity testing of *Salmonella* Pullorum, Enteritidis, and Typhimurium was assessed in one-day-old commercial broiler chicks.

MATERIALS AND METHODS

Sampling: The collected samples were intestine, liver, kidney, yolk sac and spleen with a number of 180 for each, from dead chicken embryos (n=40 cases from 4 hatcheries), dead duck embryos (n=40 cases from 4 hatcheries) (these hatcheries had hatchabilities of 83-87% due to late embryonic mortalities) and 1- to 7-day-old dead commercial broiler chicks (n=100 cases collected from 10 farms located in the Giza, Gharbia and Sharkia Governorates). Egg shells were disinfected with 70% ethyl alcohol and opened with clean scissors, through post-mortem examination was carried out, and organ samples were removed for further bacteriological examination.

Isolation and identification of *Salmonella*: Five grams of each sample were aseptically chopped into fine pieces and pre-enriched in 45 ml of tryptic soy broth for 16–20 hrs at 35–37°C. From each pre-enrichment culture 1ml was added to 10 ml of Mueller–Kauffman tetrathionate medium (Oxoid) and kept at 37°C for 24 hrs. The xylose lysine deoxycholate agar (XLD; Oxoid) was streaked with aloopful from the pre-enrichment culture, and kept for 24 hrs at 37°C. The suspected colonies of *Salmonella* were purified on XLD medium. The biochemical identification of obtained isolates was performed according to Zhang *et al.* (2013). The gained isolates were confirmed by their matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) profile according to Shehata *et al.* (2013). The isolates were serotyped by slide agglutination test after Kauffmann–White scheme using “O" and "H" antisera (Difco).

Genotypic characterisation of virulence genes: Extraction of DNA was carried out using the boiling method as previously described by Skyberg *et al.* (2006) and Tarabees *et al.* (2016). A total of seventeen virulence genes were detected using Multiplex PCR in the following 3 sets: [set 1 (amplified *cdt*B, *msg*A, *pag*C, *spi*A, and *spv*B); set 2 (amplified *inv*A, *org*A, *prg*H, *sip*B, *spa*N, and *tol*C); and set 3 (amplified *iro*N, *lpf*C, *pef*A, *sif*A, *sop*B, and *sit*C)]. Thermal conditions and reaction mixtures were used as previously described by Skyberg *et al.* (2006) and Tarabees *et al.* (2016).

Antimicrobial susceptibility testing: The susceptibility of the serotyped *Salmonella* was detected to the following antimicrobials; amoxicillin (25µg), ampicillin (10µg), chloramphenicol (30µg), doxycycline (40µg), neomycin (30µg), norfloxacin (10µg), streptomycin (10µg), sulphamethoxazole/trimethoprim (25µg) and tetracycline (30µg), using the disc diffusion method according to CLSI, (2013). The implemented media and antimicrobial discs were supplied by (Oxoid). Inhibition zones were measured to assess resistance or susceptibility. The multiple antibiotic resistance (MAR) index for each isolates was calculated by dividing the number of ineffective antimicrobials by the total number of used antimicrobials. The isolate considered to be serious if it expressed MAR index >0.2 and considered to be from a high-risk contamination origin.

Pathogenicity testing of selected isolates: The pathogenicity of selected isolates was assessed in 1-day-old commercial Hyline layer chicks (n=90). Ten birds were slaughtered at 1 day old and tested for *Salmonella*. The birds had no maternal antibodies by agglutination test and examined by bacteriologic examination to confirm absence of *Salmonella*. The chicks were divided into 4 groups, designated G1-G4. Chicks in G1 (n=20) were infected orally with 0.5 ml containing 2×10^6 CFU of *S.*Pullorum, G2 (n=20) was infected orally with 0.5 ml containing 2×10^6 CFU of *S.*Typhimurium, G3 (n=20) was infected orally with 0.5 ml containing 2×10^6 CFU of *S.*Enteritidis, and chicks in G4 were inoculated orally with phosphate-buffered saline (PBS) and considered as negative controls. All chicks were observed for mortality and clinical signs for four-weeks post-inoculation. Specimens from the liver and spleen were collected from dead birds and subjected to bacterial isolation on CASO agar. Identification was performed using a slide agglutination test and MALDI-TOF. Gross lesion scores were assessed according to Matsuda *et al.* (2011).

There was approval to perform this study from the Committee of Animal Ethics of the Faculty of Veterinary Medicine, University of Sadat City as the steps complied with the Guidelines for the care and utilization of animals during research.

RESULTS

Isolation and identification of *Salmonella* enterica serovars: All the obtained 18 isolates were confirmed to be *Salmonella* enterica after implementing conventional and MALDI-TOF methods. The MALDI-TOF spectra of *S.*Pullorum, *S.* Enteritidis and *S.* Typhimurium were explained in (Fig. 1). The isolates were serologically confirmed based on "O" and "H" antisera and the gained serotypes were listed in (Table 1). The percentages of

Salmonella-positive samples were 7, 12.5 and 15% for broilers, dead chicken embryos, and dead duck embryos, respectively. Only three (3/10) commercial broiler chicken farms were positive for *Salmonella*. *S.* Typhimurium (n=3) and *S.* Enteritidis (n=4) were isolated from commercial broiler chickens. *S.*Enteritidis (n=1), *S.*Amsterdam (n=1), *S.*Vejle (n=1), *S.*Pietersburg (n=1), and *S.*Atakpame (n=2) were isolated from dead embryos collected from broiler hatcheries. Moreover, *S.* Infantis (n=1), *S.* Vejle (n=3) and *S.* Enteritidis (n=1) recovered from dead duck embryos.

Virulence gene profiles: The isolated *Salmonella* serotypes had different frequencies of target genes (Table 2). The most predominant virulence genes were *inv*A, *cdt*B, *spa*N, *org*A, and *iro*N, which were found in 17 (94.4%), 15 (83.3%), 14 (77.7%), 13 (72.2%) and 12 (66.7%) of the isolates, respectively. The *sip*V, *lpf*C, *sop*B, *prg*H, and *sit*C were successfully amplified in 7 (38.8), 7 (38.8%), 7 (38.8%), 5 (27.7%) and 3 (16.6%) *Salmonella* serotypes, respectively. All *S.* Enteritidis (6/6) were positive for *inv*A, *iro*N, *lpf*C, and *sop*B moreover, all *S.* Typhimurium (2/2) were positive for *inv*A, *cdt*B, *sip*V, *spa*N, *org*A, and *iro*N. *S.* Pullorum isolates were positive for *inv*A, *cdt*B, *spv*B, *sip*V, *spa*N, *org*H, and *iro*N.

Antimicrobial susceptibility testing: The isolated *Salmonella* enterica serotypes were resistant to one or more antimicrobials (Tables 2, 3). The tested *Salmonella* isolates showed antibiotic resistance to ampicillin, neomycin, sulphamethoxazole/trimethoprim, streptomycin, tetracycline, chloramphenicol, doxycycline and norfloxacin at rates of 94.4, 88.9, 72.2, 61.1, 44.4, and 33.3%, respectively. The MAR index analysis (Table 3) revealed that five isolates of *S.* Enteritidis had very high MAR index values (0.50 to 1.0). *S.* Typhimurium exhibited MAR index values from (0.75 to 0.87). *S.* Pullorum had an MAR index of 0.5. Other Salmonella isolates showed variable MAR indexes ranging from (0.12 to 0.87).

Pathogenicity: Only *S.* Pullorum gave higher mortality 7/20 (35%) and higher lesion scores in liver and spleen (being enlarged and contained necrotic foci) when compared with the lesion scores of *S.*Enteritidis and *S.* Typhimurium (Table 4). Moreover, the *S.* Pullorum was recovered from the organs of dead chicks on specific media. Additionally, the existence of *S.* Pullorum was confirmed by slide agglutination test and MALDI-TOF profile.

DISCUSSION

Salmonella is a major foodborne pathogen responsible for many infectious diseases in animals and humans worldwide (Raguenaud *et al.,* 2012: Manoj *et al.,* 2015). In this study, a total of 8 *Salmonella* serotypes were isolated from Egyptian commercial broilers, dead chickens, and duck embryos. The data demonstrated that *S.* Typhimurium and *S.* Enteritidis were isolated from 1- to 7-day-old commercial broilers which agreed with El-Sharkawy *et al.* (2017). Hatcheries are major sources of

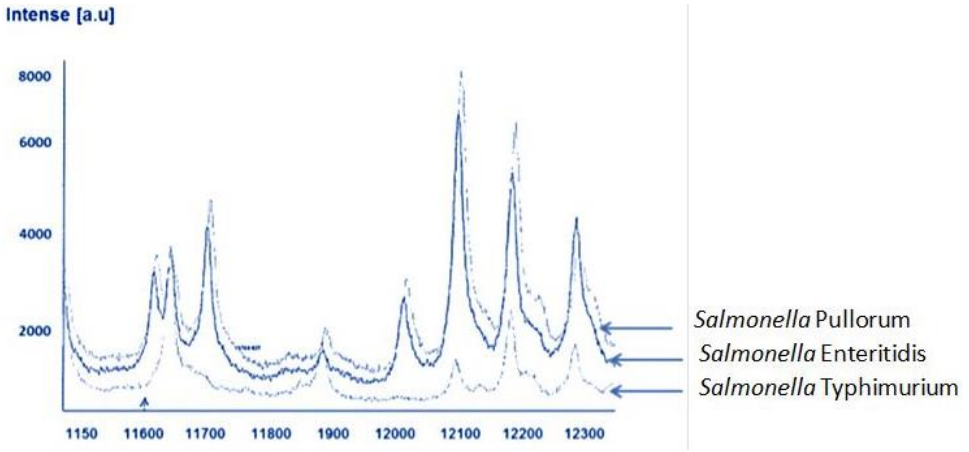

Fig. 1: MALDI-TOF spectra of *S.*Pullorum, *S.*Enteritidis and *S.*Typhimurium

Table 1: Rates of *Salmonella* serotypes isolated from hatcheries and poultry farms

Type and number of cases	Type and number of samples	*Salmonella*-positive		Antigen			Total (%)
		Serotype	Freq.	O	H		
					Phase 1	Phase 2	
Commercial broilers (100)	Intestine (100)	*S.* Pullorum	1	1,9,12	-	-	7/100 (7%)
	Kidney 100)	*S.* Typhimurium	1	1,9,12	1	1,2	
		S. Enteritidis	1	1,9,12	g.m	-	
	Liver (100)	*S.* Typhimurium	1	1,9,12	1	1,2	
		S. Enteritidis	1	1,9,12	g.m	-	
	Spleen (100)	*S.* Enteritidis	1	1,9,12	g.m	-	
	Yolk sac (100)	*S.* Enteritidis	1	1,9,12	g.m	-	
Dead chicken embryos (40)	Intestine (40)	*S.* Enteritidis	1	1,9,12	g.m	-	5/40 (12.5%)
	Kidney (40)	*S.* Amsterdam	1	3,{10}{15}{15, 34}	g,m,s	-	
	Liver (40)	*S.* Vejle	1	3,{10}{15}	e,h	1,2	
	Spleen (40)	*S.* Pietersburg	1	3,{10}{15, 34}	Z69	1,7	
	Yolk sac (40)	*S.* Atakpame	1	8,20	e,h	1,7	
Dead duck embryos (40)	Intestine (40)	*S.* Infantis	1	6,7,14	R	1,5	6/40 (15%)
	Kidney (40)	*S.* Vejle	2	3,{10}{15}	e,h	1,2	
	Liver (40)	*S.* Enteritidis	1	1,9,12	g.m	-	
	Spleen (40)	*S.* Vejle	1	3,{10}{15}	e,h	1,2	
	Yolk sac (40)	*S.* Atakpame	1	8,20	e,h	1,7	18/180 (10%)

Table 2: Frequency of virulence genes by multiplex PCR, phenotypic antimicrobial resistance profiles, and multiple antimicrobial resistance index (MAR) of *Salmonella* serovars

Type of samples	*Salmonella* Serotypes	No. of sero-types	Virulence genes																	Phenotypic antimicrobial resistance	MAR Index
			*inv*A	*spi*A	*pag*C	*cdt*B	*msg*A	*spv*B	*sip*V	*prg*H	*spa*N	*org*A	*tol*C	*iro*N	*sit*C	*lpf*C	*sif*A	*sop*B	*pef*A		
Commercial broilers	*S.* Typhimurium	2	+	-	-	+	-	-	+	+	+	+	-	+	+	-	-	-	-	AM 10, C 30, DO 10, N30, S10, TE	0.75
			+	-	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-	AM 10, C 30, DO 10, N30, S10, TE, SXT	0.87
	S. Pullorum	1	+	-	-	+	-	+	+	-	+	+	-	+	-	-	-	-	-	AM 10, N30, S10, SXT	0.50
	S. Enteritidis	4	+	-	-	+	-	+	+	+	+	+	-	+	+	+	-	+	-	AM 10, C 30, N30	0.50
			+	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	-	AM 10, C 30, DO 10, S10, SXT and N30	0.75
			+	-	-	-	-	-	-	+	-	-	-	+	-	+	-	+	-	AM 10, C 30, N30, S10, SXT	0.62
			+	-	-	+	-	-	-	+	-	-	-	+	-	+	-	+	-	AM 10, C 30, N30, NOR, S10, SXT	0.72
Dead chicken embryos	*S.* Enteritidis		+	-	-	+	-	-	+	-	-	-	-	+	-	+	-	+	-	SXT	0.12
	S. Amsterdam	1	-	-	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	AM 10, C 30, DO 10, N30, NOR, SXT, TE	0.87
	S. Vejle	1	-	-	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	AM 10	0.12
	S. Pietersburg	1	+	-	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	AM 10, N30, NOR, S10, TE	0.62
	S. Takpame	1	+	-	-	+	-	+	-	-	+	+	-	+	-	-	-	-	-	AM 10, DO 10, N30, SXT	0.5
Dead duck embryos	*S.* Infantis	1	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	AM 10, N30, SXT	0.37
	S. Vejle	3	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	AM 10, N30, SXT	0.37
			-	-	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	AM 10, N30, NOR, S10, SXT, TE	0.75
			-	-	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	AM 10, N30, NOR, S10, TE	0.75
	S. Enteritidis	1	+	-	-	+	-	-	-	-	+	-	-	+	-	+	-	+	-	AM 10, C 30, DO 10, N30, NOR, S10, SXT, TE	1
	S. Atakpame	1	+	-	-	+	-	-	+	-	+	+	-	+	-	-	-	-	-	AM 10, DO 10, N30, S10, SXT	0.62
Total			13/18 (72.2%)	0/18 (0.0%)	0/18 (0.0%)	15/18 (83.3%)	0/18 (0.0%)	0/18 (0.0%)	7/18(38.8%)	5/18 (27.7%)	14/18 (77.7%)	12/18 (66.7%)	0/18 (0.0%)	17/18 (94.4%)	3/18 (16.6%)	7/18 (38.8%)	0/18 (0.0%)	7/18 (38.8%)	0/18 (0.0%)		

Salmonella contamination (Martelli *et al.*, 2016). The data revealed that *S.* Enteritidis, *S.* Amsterdam, *S.*Vejle, *S.* Pietersburg, and *S.* Atakpame were isolated from dead chicken embryos collected from broiler hatcheries. *S.* Infantis, *S.* Vejle and *S.* Enteritidis were isolated from dead duck embryos. Contamination of duck hatcheries with different *Salmonella* serovars has been previously confirmed in different duck hatcheries with a hatchability rate of 83-87% in the UK by Martelli *et al.* (2016). Although other factors are associated with low hatchability, decreased egg hatchability has been attributed to *Salmonella* infection (Hameed *et al.*, 2014). Transmission of *Salmonella* during incubation leads to pipped or unpipped chicken eggs. *Salmonella* reaches embryos vertically or horizontally during hatching (Bailey *et al.*, 1994: Hameed *et al.*, 2014). From the isolation results, *S.* Infantis was isolated from dead duck embryos. This serotype is not host-associated and has zoonotic importance. There is an epidemiological association between *S.*Infantis isolates of broiler and human origin was previously reported (Miller *et al.*, 2010).

The severity of *Salmonella* infection is primarily mediated by an array of virulence-encoding genes. The majority of *Salmonella* virulence markers, like adhesion, invasion, intracellular proliferation and toxin genes, located on *Salmonella* pathogenicity islands (SPIs) present either on the chromosome or the plasmid (Miller *et al.*, 2010). There are two main SPI areas, the first contains invasion genes, while the second is essential for intracellular pathogenesis and plays a crucial role in systemic infections caused by *Salmoella*. In this work, an array of virulence genes (a set of seventeen genes) were screened in *Salmonella* serovars isolated from poultry in Egypt using multiplex PCR. *inv*A, *org*A, *sop*B, *prg*H,

Table 3: Antibiogram phenotypic patterns

Antibiotics	Antibiogram phenotypic pattern					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Tetracycline	10	55.6	0	0	8	44.4
Ampicillin	1	5.6	0	0	17	94.4
Neomycin	2	11.1	0	0	16	88.9
Streptomycin	3	16.7	4	22.2	11	61.1
Doxycycline	11	61.1	1	5.6	6	33.3
Chloramphenicol	9	50	1	5.6	8	44.4
Amoxicillin	8	44.4	0	0	10	55.6
Sulphamethoxazole/trimethoprim	2	11.1	3	16.7	13	72.2
Norfloxacin	11	61.1	1	5.6	6	33.3

Table 4: Mortality and gross lesion scores of chickens infected with *Salmonella* serovars

Group	Challenge	Mortality	Liver		Spleen	
			Enlarge-ment	Necrotic foci	Enlarge-ment	Necrotic foci
G1	*S.* Pullorum	7/20 (35%)	3.4	2.6	2.4	2.5
G2	*S.* Enteritidis	0	2.5	2.3	1.2	1.4
G3	*S.* Typhimurium	0	1.6	1.8	1.7	1.5
G4	Control	0	0	0	0	0

*lpf*C, *pef*A, and *tol*C are virulence genes that have roles in host recognition and invasion. The *sit*C and *iro*N have roles in iron acquisition (Skyberg *et al.,* 2006; Han *et al.,* 2013; Tarabees *et al.,* 2016). The data showed that *iro*N was detected in 94.4% of the tested strains, while *sif*A, which is required for the formation of the filamentous structure (Brumell *et al.,* 2002), was not amplified in this study. Both the *sip*B and *spa*N genes play roles in the entry mechanism to non-phagocytic cells and killing of macrophage (Chen *et al.,* 1996). The *spa*N was amplified in 77.7% of isolates, while *sip*B was not amplified in this study. The data showed that, *inv*A was successfully detected in 95% of the isolated *Salmonella*, remarks the value of *inv*A a key marker in the molecular diagnosis of *Salmonella* in poultry (Dong *et al.,* 2011). *Salmonella* outer proteins (*sop*) with other effector proteins have a role in its pathogenesis (O'Regan *et al.,* 2008) and its production is mainly governed by an array of *sop* encoding genes (*sop*A-E). In this study, *sop*B was detected in only Salmonella enterica serovars Enteritidis (6 isolates) and Infantis (1 isolate). This outcome is inconsistent with the results of Gharieb *et al.* (2015), who stated that, *sop*B is successfully amplified only in 2 untypable isolates and 1 isolate *S.* Typhimurium. In this study, all *S.* Enteritidis isolates harbor *sop*B, which may signify its value in pathogenesis.

It has been suggested that chickens are a source for multidrug-resistant *Salmonella* serotypes in humans (Eguale, 2018). In the present study, most of the tested *Salmonella* serovars were multi-drug resistant and had high MAR indices against the commonly used antibiotics in the poultry industry in Egypt. The data showed that, 94.4%, 88.7% and 72.2% of the tested *Salmonella* serotypes were resistant to ampicillin, neomycin, and sulphamethoxazole/ trimethoprim. This outcome is inconsistent with the data of El-Sharkawy *et al.* (2017) who found that all the tested *S.*Typhimurium and untypable isolates were resistant to ampicillin. Monitoring of the isolated salmonellae from poultry and hatcheries for their antibiotic resistance profile will provide useful information in establishing preventive and curative measures to control *Salmonella*-induced infection in poultry and humans.

The genetically confirmed *S.* Enteritidis, *S.* Typhimurium, and *S.* Pullorum were selected to evaluate their pathogenicity in one-day-old commercial layer chickens. Although *S.* Enteritidis and *S.* Typhimurium did not cause any mortality in the infected chickens, infection resulted in mild pathogenicity residues based on the post-mortem examination. Conversely, chickens infected with *S.*Pullorum showed a mortality rate of 35%, and the main clinical signs were whitish chalky diarrhea and depression. The post-mortem examination revealed the presence of necrotic foci on the enlarged liver, enlarged kidneys, the presence of a whitish caecal core and accumulation of urates in the two ureters. *S.*Pullorum is one of the foremost causes of mortality among poultry (Campioni *et al.,* 2012). A comprehensive understanding of the pathogenesis of *S.*Pullorum will help minimize the severe economic losses usually associated with this infection. In addition, the data herein will help pave the road towards an effective *Salmonella* vaccine that is safe for use on large-scale farms.

Conclusions: The isolation of non-typhoidal *Salmonellae* from dead embryos in chicken and duck hatcheries, as well as from commercial broiler baby chicks, affirms the value in maintaining hatchery and farm biosecurity standards. Although this is the first report regarding *Salmonella* in Egyptian hatcheries, the role of hatcheries in the persistence and transmission of *Salmonella* infection and the reduction of hatchery contamination should be studied in detail. Additionally, the high occurrence of MAR in *Salmonella* isolates highlights the potential for human infection with multi-drug-resistant *Salmonella*.

Authors contribution: AS, HS, AAE, MSAEE, RT and MK were the leaders of this research work, they stated the work plan, observed and evaluated the work steps. They also aided in the identification, the serotyping, the genotyping, the antimicrobial susceptibility testing, the data analysis and manuscript writing and editing. ST, IM, AS and WA helped in the in the study conceptualization, provided some technical a device and data analysis.

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